LIGHT DIFFRACTION BY SINGLE STRIATED MUSCLE FIBERS†

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In two preceding reports (Meyer-Arendt, 1957a, b), the scattering of light has been discussed that originates from single tissue cells or cell componenets. Two sets of information can be obtained by this microscattering technic: first, information about the turbidity of the sample shown by a more diffuse scattering; secondly, information about any regularity, if present at all, in the arrangement of the underlying structures. For these studies two narrow and almost parallel light beams were projected into the test specimen. One of them was directed onto the cell; the other served as a reference. The scattered light has been observed through an ordinary, high power microscope, at a plane kept in a constant distance of 106 μ above the specimen.

The objective of the investigations presented here is to study light diffraction patterns effected by striated muscle fibers. In particular, such patterns were attempted to obtain from minute portions within one single muscle fiber. Similar measurements have been done by Buchthal and Knappeis (1940), while earlier Ranvier (1874) and later-on Neumann (1951) operated in a macroscopic range, using whole sections through muscle tissue.

TECHNIC

The technic used has been essentially the same as reported previously. Additionally, however, following a suggestion by S. Inoué, the interference patterns in the back focal plane of the microscopic objective were recorded.

Light of 546.1 mμ wavelength is obtained from a high pressure mercury lamp and directed into a pinhole 125 μ in diameter. An image of this pinhole is projected, by an aplanatic objective of 50 mm focal length, into the muscle fiber under investigation. By closing down the iris diaphragm of the aplanate to f : 18, faint lateral maxima can be saved from being overlighted by the intense zero order light beam. Since most diffraction maxima were fairly distinct, no reference beam was needed. A Zeiss planachromatic oil immersion objective, x 100, was used for observing the pattern. The interference patterns in the back focal plane of this objective were recorded through an auxiliary microscope (telescope), as used for aligning phase contrast objectives. Photomicrographs were taken by an attachment camera on Kodak Process Ortho Sheet Film.

RESULTS

Striated muscle fibers are built of alternating lighter "I," and darker and denser "A" discs, the A bands having a higher refractive index. In the investigations reported, around 4 to 5 A and I bands were covered by the incident light beam, thus causing diffraction patterns from this number of periods. Specimens stained either with iron-hematoxylin or hematoxylin-eosin were used. The diffraction patterns seen in the back focal plane are substantially similar to those observed previously between specimen and objective. They consist essentially of a

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series of maxima, the arrangement of which is perpendicular to the direction of the bands within the muscle fiber (fig. 1). The distances between the maxima are related to the spacing of the bands. Perpendicular to these maxima, another series of faint maxima can be recognized. These are caused by the fibrillar structure of the muscle fiber. It may be of interest to note that these latter maxima have not been recorded before. This may be explained by the fact that these maxima are faint and of very low light intensity, compared with the distinct maxima caused by the cross striations. Even those are not easily recorded.

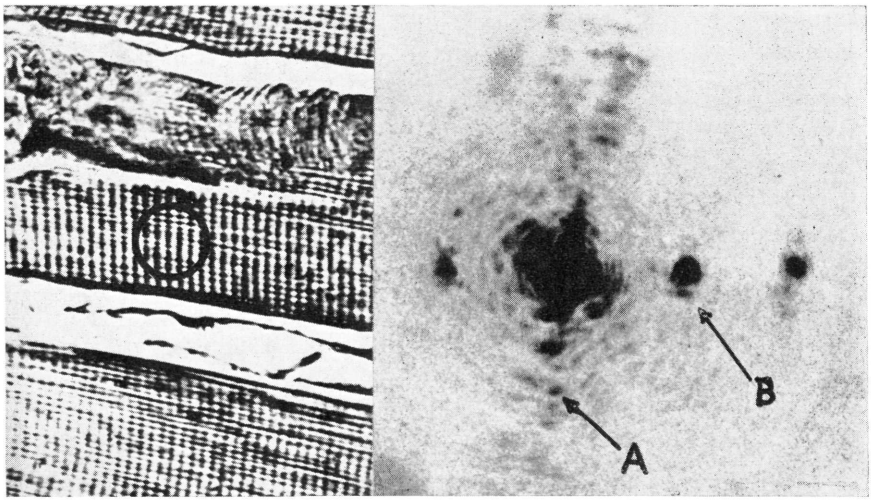

FIGURE 1. Left: Photomicrograph of human striated muscle fibers. Magnification about x 750. The encircled area designates the size of the incident light beam. Right: Primary interference pattern from an area as shown at left, recorded from the back focal plane of the microscopic objective. A—fibrils; B—cross striations.

It can be shown, thus, that light diffraction patterns can be obtained from minute areas within one single muscle fiber. Such patterns seem to show more details than have been reported previously.

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